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Using Sesame Meal Protein Hydrolysates With Pepsin As An Alternative Nitrogen Source In Some Culture Media For Growing Microorganisms

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Abstract
The study aimed to use defatted sesame meal as a rich protein source, exploiting its high protein content, extracting and enzymatically analyzing it, and using the resulting protein hydrolysates as an alternative to expensive nitrogen sources in some culture media for growing specific microorganisms. The extraction process was carried out using 70% ethanol, and the amino acids were identified using HPLC. The results showed that the highest amino acid percentages were glutamic acid (14.15%), arginine (11.25%), and aspartic acid (6.15%), while essential acids such as leucine (5.62%), lysine (2.68%), and methionine (2.36%) recorded lower percentages. Enzymatic hydrolysis of the protein was performed using pepsin, with the highest degree of hydrolysis reaching 37% after 180 minutes. The highest solubility was recorded at pH = 11, while the highest water holding capacity reached 2.9 g/g at pH = 7.
The protein hydrolysates were used in the preparation of Nutrient agar and MacConky agar media after replacing peptone as a nitrogen source with these hydrolysates. The numbers of Bacillus subtilis and Staphylococcus aureus reached (167, 150, 158) and (205, 185, 190) colony forming units (CFU) × 10^5 CFU, when using commercial and modified media after 180 and 240 mins, respectively. However, the numbers of Escherichia coli and Salmonella typhi reached (175, 150, 162) and (155, 132, 143) × 10^5 CFU. The efficiency of these decomposers in stimulating the growth of Aspergillus niger and Aspergillus flavus was evaluated using commercial and modified Sabouraud Dextrose Agar media. The growth diameters of A. niger were recorded at (535, 525, 530) mm, and those of A. flavus at (450, 430, 440) mm. The results indicated the effectiveness of the protein hydrolysates produced from sesame meal in supporting the growth of microorganisms, confirming their potential as an alternative and effective nitrogen source in the preparation of culture media.
Key words: *sesame meal, protein hydrolysates, microorganisms.*

INTRODUCTION

Culture media are used in microbiology laboratories to grow, isolate, purify, study, and preserve various types of microorganisms, as well as to produce primary and secondary metabolites. The components of culture media vary depending on the nutritional requirements of microorganisms, which differ in their properties and physiology. With the discovery of new types of microorganisms, the number of culture media produced by specialized companies is increasing. Despite the differences between the components of culture media, they all share the need for a carbon source, such as carbohydrates and nitrogen, such as peptone, yeast extract, meat extract, growth factors, buffers, and selective elements (Bonnet et al., 2020; Elizabeth et al., 2023).
Nitrogen is one of the essential components of living cells. It is a component of nucleic acids, proteins, and bacterial cell walls, and constitutes 10–15% of the total. The dry weight of a bacterial cell. Microorganisms obtain nitrogen from inorganic and organic sources found in various microorganism growth media.

Peptone is one of the most important and expensive organic nitrogen sources found in culture media. Peptone varies depending on its source, peptide chain length, the type of amino acids it contains, and its preparation method. It may be enzymatic, using different types of enzymes from different sources, or chemical, using different bases and acids. This affects its availability to microorganisms and its biological and functional properties, such as antioxidant activity, solubility, water-holding capacity, emulsification, color, and other properties (Villamil et al., 2017; Yasmin and Al-Shamary, 2024).
Rout et al. (2018) indicated that sesame seeds are rich in fats, proteins, minerals, vitamins, and dietary fiber. Studies have shown that sesame seeds contain 21.9% protein. 61.7% is fat. In addition, to being rich in essential minerals such as calcium, iron, manganese, phosphorus, magnesium, selenium, and copper, these minerals play a vital role in bone mineralization and health. Due to their high nutritional value, sesame seeds are known as the comprehensive nutrient bank and the crown of precious grains.
Sesame protein contains a variety of proteins, primarily globulin, translucent protein, alcohol protein, and glutenin. Of these, globulin has the highest content, while alcohol protein has the lowest content (Cui et al., 2021; Fuji et al., 2018). Given the importance of peptone as a nitrogen source in preparing culture media for microorganisms, the current study aimed to exploit the high protein content of sesame seed oil extraction waste, analyze it enzymatically, and use it as an alternative nitrogen source in some culture media for microorganisms.

MATERIALS AND METHODS

Preparation of Sesame Meal Powder

Sesame was purchased from local markets in Baghdad, Iraq between July, 10 and August, 22. Impurities were removed, and the seeds were pressed using a Yoda press to extract the oil and produce the sesame meal. The sesame meal was stored in bags until use.

Fat Removal

The method described by (Poveda et al., 2016) was used to remove fat from sesame meal powder. Sesame meal powder was mixed with hexane at a ratio of 10 w/v (10 w/v) under continuous stirring for 5 hours using a magnetic stirrer. The mixture was then left to stand for 24 hours at room temperature. It was then filtered using filter paper and stored at 4°C until use.

ESTIMATING THE CHEMICAL COMPOSITION OF DEFATTED SESAME

Estimating the Moisture Percentage:

The moisture percentage in the sesame powder was estimated according to the method described in (AOAA, 2010), by weighing 3 g of the sample and placing it in a drying oven at 105°C until the weight stabilized. The moisture percentage was calculated after estimating the weight of the sample and the drying vessel before and after drying using the following equation:
Moisture Percentage% = Weight of the sample before drying – Weight of the sample after drying x 100.

Estimating the Ash Percentage

The ash percentage in the sesame powder was estimated according to the method described in (AOAC, 2010), by weighing 3 g of the sample and placing it in an incineration oven at 225°C for 16 hours. The ash percentage was calculated after estimating the weight of the sample and the incineration vessel before and after incineration using the following equation:

Ash% = (weight of residue x 100)/(weight of Sample)

Protein Percentage Estimation

The protein percentage in sesame meal powder was estimated using the Kjeldahl method described in (Dijk, 2000) as follows:

0.5 g of sample was placed in a digestion flask, with an appropriate amount of Kjeldahl catalyst added. 5 ml of sulfuric acid was then added, and a control (blank) treatment was prepared from the same materials mentioned above without adding the sample.

The digestion process was then carried out by placing the digestion tubes in a heating unit and gradually increasing the temperature until reaching 420°C. After the sample turned light blue, it was allowed to cool to room temperature. Distillation was then carried out in a flask containing a 40% sodium hydroxide solution, followed by the addition of a 20% boric acid solution. The solution was placed under a condenser, ensuring that the tip of the condenser was immersed in the solution. Drops of the reagent solution were then added. The titration was carried out using 0.1 M hydrochloric acid solution with 25 ml of distillate, and the titration continued until the solution turned pink. The protein percentage was calculated using the following equation:

$$\text{Protein } \% = ((b-a) \times 0.1 \times 14 \times 100 \times 6.25)/(w \times 1000)$$

Estimating the Lipid Percentage

The lipid percentage in the sesame seed meal powder was estimated according to the method described in (AOAC, 2010). 10 grams of the sample was weighed and placed in a Soxholet lipid extraction device. 250 ml of hexane was added. The extraction process continued for 5 hours. The solvent was collected from the device, and the temperature in the beaker was raised to 80°C to ensure the evaporation of the solvent residue. The lipid percentage was estimated according to the following equation:

$$\text{Lipid } \% = (\text{weight of lipid} \times 100)/(\text{weight of sample})$$

Estimating the Carbohydrate Percentage

The carbohydrate percentage in the sesame seed meal powder was estimated according to the method described in (AOAC, 2010). Described in (FAO, 2003) from the following equation:

$$\text{Carbohydrate } \% = 100 - (\text{Moisture} + \text{Protein} + \text{Ash} + \text{Lipid})$$

Protein extraction from defatted sesame meal powder

Protein was extracted according to the method described by Kadhim & Shakir (2019). The defatted sesame meal powder prepared in paragraphs 2-3 was mixed with 70% ethanol at a ratio of 10:1, stirring continuously for two hours using a magnetic stirrer. The sample was then centrifuged 5,000 times at the ground acceleration for 15 minutes. The precipitate was then collected, and the sample was dried and stored until use.

Estimation of amino acids in the extracted protein

The amino acids in the protein extracted from defatted sesame prepared in paragraph (2-3) were determined according to the method described by (Cvjetkovic et al., 2019) using a high-performance liquid chromatography (HPLC) machine, type HPLCSYKAM, model LC-2010A HT, using a C18ODS column measuring (25 cm x 4.6 mm) at a flow rate of 1.2 ml/min. The column temperature was 40°C. 100 mg of the prepared sample was mixed with 15 ml of 0.5 M sodium chloride solution and 0.01 ml of 0.05 M 4KH2PO4 solution. The sample was mixed using a magnetic stirrer for 10 minutes, and centrifuged at 7000 g/min. The ground was left for 20 minutes, the sample was filtered, and then injected into the device. 3-6 Preparation of Pepsin-Enzyme Protein Hydrolysates.

The hydrolysates were prepared according to the method described by Liu and Chiang (2008), with some modifications. The extracted protein was mixed with 0.1 N hydrochloric acid solutions, at a ratio of 20:1 (w/v), and the mixture was incubated at 5°C for 1 hour. The pH was then adjusted to 2 using 0.1 N sodium hydroxide solutions. The mixture was placed in a 37°C water bath for 15 minutes. Then, pepsin was added at a rate of 5,000 units per 1 g of extracted protein, according to the manufacturer's instructions.The samples were placed in a shaking incubator at 37°C. Samples were withdrawn after 30, 60, 120, 180, and 240 minutes of hydrolysis. They were then placed directly in an 85°C water bath for 15 minutes to inactivate the protein. Enzyme activity was assessed, and the samples were then dried and stored until use.

Degradation degree assessment
The method described by Rahmi et al., (2022) was used to assess the degree of degradation. 2 ml of the degraded products were mixed with 2 ml of a 20% (w/v) TCA solution in 10 ml tubes. The mixture was left for 30 minutes, after which it was centrifuged at 5000 times the ground acceleration for 30 minutes. The Kjeldahl method was used to estimate the percentage of protein in the mixture and the supernatant, and the degree of hydrolysis was calculated according to the following equation:

DH% = (10% TCA soluble nitrogen in the sample)/(Total nitrogen in the sample) x 100

Solubility Estimation
The method described by Catterjee et al. (2015) was followed to estimate the solubility of pepsin hydrolysates. 0.1 N hydrochloric acid solution and 0.1 N sodium hydroxide solution were used to adjust the pH of the protein hydrolysate solution to (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12). The solutions were then mixed for 30 minutes at 150 rpm, followed by centrifugation at 10,000 g/min for 15 minutes. The supernatant was collected, and the percentage of protein was estimated using the Kjeldahl method. The percentage solubility was calculated using the following equation:

Solubility % = (protein content in the supernatant)/(total protein content in the sample) X 100

Estimation of the water-holding capacity of sesame protein enzymatic hydrolysates
Water-holding capacity was estimated according to the method described by (Onsaard et al., 2010).Procedure: 1 g of the hydrolyzed powder that showed the best degree of dissolution and solubility was dissolved in 20 ml of distilled water and mixed for 5 minutes. The pH was adjusted to (5, 6, 7) and left for 15 minutes at room temperature. The process was centrifuged at 10,000 g/min for 10 minutes. Water-holding capacity was defined as the weight of water absorbed by 1 g of protein hydrolysates. Water holding capacity is determined according to the following equation:

Water holding capacity = weight of water added to the sample - weight of water after centrifugation / weight of the sample × 100

Extracted sesame protein yield

The yield was calculated based on the method used by Jaziri et al. (2020). The sesame and the protein concentrate extracted from it were weighed. The yield was calculated according to the equation below:

$$Yield\% = Weight\ of\ extract\ or\ protein\ concentrate\ / Weight\ of\ raw\ material \times 100$$

Determination of Protein Species in Defatted Sesame Protein Isolate

Proteins in defatted sesame protein powder were determined according to the method described by (Cvjetković et al., 2019). 100 mg of the sample was extracted to determine albumin and globulin content using 15 ml of NaCl solution with 0.01 g of KH_2PO_4 (pH = 7.6) at room temperature. After removing the supernatant, gliadins were extracted using a mixture of ethanol, 1-propanol, and 2-propanol under the same conditions. Samples were homogenized on a Vortex for 2 minutes, followed by magnetic stirring for 10 minutes.Centrifugation was performed for 15 minutes (for albumin and globulin) and 20 minutes (for gliadins) at 7,000 rpm. The floating layers were collected, and the samples were filtered using a 0.45 µm membrane before analysis. HPLC analysis was performed using a SYKAM (Germany) device to quantify protein.

USE OF PEPSIN PROTEOLYTICS AS AN ALTERNATIVE TO A NITROGEN SOURCE IN THE PREPARATION OF SOME CULTURE MEDIA

Pepsin proteolytics, which yielded the highest solubility after a lysis time of 180 and 240 minutes, were used as an alternative to a nitrogen source in the preparation of some culture media, including Nutrient Agar, MacConky Agar, and Sabouraud Dextrose Agar. These media were used to grow Gram-positive and Gram-negative bacteria and molds, including *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli, Salmonella typhi*, *Aspergillus niger*, and *Aspergillus flavus*, respectively. These media were all obtained from the Graduate Biotechnology Laboratory, College of Agricultural Engineering Sciences.

All commercial media were prepared according to the manufacturer's instructions. Alternative media were prepared by replacing the peptone nitrogen source in each medium with pepsin proteases after 180 and 240 minutes of lysis, respectively. All culture media were autoclaved at 121°C and 15 psi for 15 minutes.

Bacterial Growth on Commercial and Alternative Culture Media

The poured-plate method described by (Ahmed and Al-Shamary, 2019) was used to grow different bacterial species after performing the required decimal dilutions. 1 ml of each dilution of the different prepared bacterial species was transferred to Petri dishes. Commercial Nutrient Agar and its alternative medium were poured separately into the dishes containing *Bacillus subilis* and *Staphylococcus aureus*, mixed well, and allowed to solidify. The dishes were then incubated in an incubator at 37°C for 48 hours. Commercial MacConky Agar and its alternative medium were poured separately into the dishes containing *Salmonella typhi* and *Escherichia coli*, mixed well, and allowed to solidify. The dishes were then incubated in an incubator at 37°C for 48 hours. Colony counts were calculated using a colony counting device.

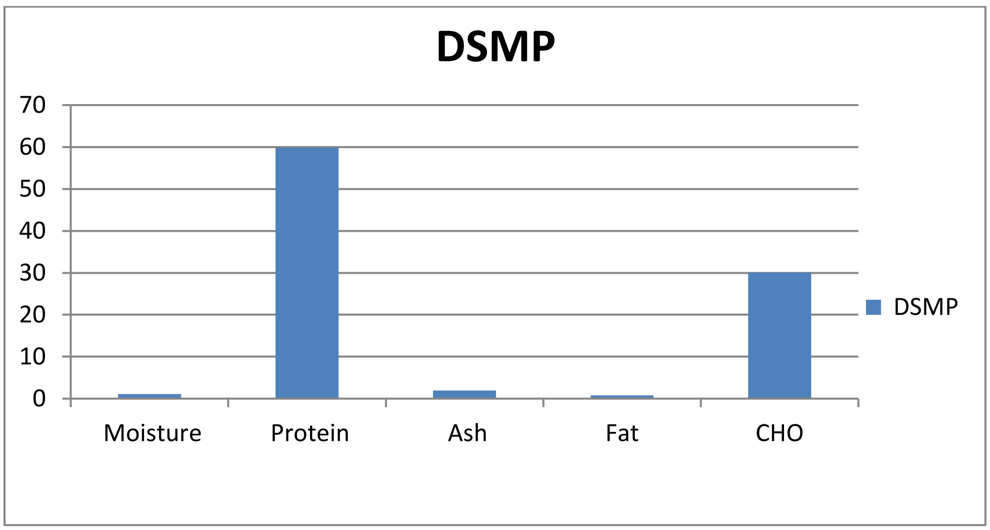
Mold Growth on Commercial and Alternative Media:

Commercial Sabouraud Dextrose Agar and its alternative media were poured into Petri dishes and allowed to solidify. A uniform piece of *Aspergillus flavus* and *Aspergillus niger* fungal growth was transferred from the activated cultures to the center of the plates containing the solid media. The cultures were incubated at 30°C for 72 hours, and the results were recorded by calculating the diameter of the fungal growth.

RESULTS AND DISCUSSION

Chemical Composition

Figure (1) shows the chemical composition of defatted sesame meal powder (DSMP). The percentages of moisture, protein, ash, fat, and carbohydrates in DSMP were (1.08, 59.8, 1.90, 0.8, and 30.04)%, respectively. The results show a high protein content in defatted sesame meal powder compared to other components, which encourages its investment and use as a source of protein and amino acids in various fields, including its use as an inexpensive alternative source of nitrogen in culture media used in growing various types of microorganisms.



The fat removal process reduced the fat content, significantly reducing the fat content to 0.8% in defatted sesame seed meal, which led to an increase in the protein content from 35.5% to 59.8%. These results were similar to several other results. Mathews et al. (2022) reported that sesame seed meal contained moisture, protein, ash, fat, and carbohydrates (4.19, 41.19, 6.15, 8.64, and 38.18%), respectively. However, the percentages of defatted sesame seed meal were (0.89, 80.05, 2.4, 0.25, and 6.65%), in the same order. Shamurad et al. (2019) also indicated that removing oil from sesame seeds significantly increases the protein concentration. The difference in the chemical composition of sesame is due to the agricultural conditions and climate, as well as the different types of seeds, which also lead to differences in the chemical composition of the seed and the method of oil extraction (Khan and Memon, 2022).

Sesame Protein Yield

The extract yield reached 81% of the protein from the defatted sesame powder, which is a relatively high yield. Al-Rubaie (2025) indicated that the protein yield obtained depends on the concentration of the material used in the extraction process, the temperature, the time, and the extraction method. Yüzer et al. (2023) found that the yield of the isolated sesame protein reached 88.98%.

Protein Estimation in the Extracted Defatted Sesame Powder

The results of Table (1) showed that gluten protein was the most abundant protein in the defatted sesame, constituting 41% of the total protein. Gluten is classified as a protein that is insoluble in water and saline solutions. It is often associated with albumin and cell walls. Albumin, a water-soluble protein with a high content of essential amino acids, accounts for 19.32% of the protein, giving it a high nutritional value. It also contributes to osmotic pressure regulation and cellular transport within plants. Globulin, on the other hand, accounts for 9.07%. It is a protein that is soluble in saline solutions. The variation in the proportions of these proteins reflects their different physiological and functional properties, confirming that sesame is a rich source of nutritionally valuable proteins, supporting its use in food and industrial fields.

Table (1) Protein Estimation in the Extracted Defatted Sesame

Protein	**Con%**
Albumin	19.32
Globulin	9.07
Gluten	41.0

These results are agreed with (Sa et al., 2022), which determined the protein contents in sesame meal as follows: gluten (47.42%), albumin (22.84%), and globulin (10.52%).

Estimation of Amino Acids in Sesame Meal Powder

Table (2) shows the amino acid composition of the protein extracted from sesame meal. It is noted that there is a high percentage of the amino acid glutamic acid, followed by arginine, aspartic acid, lucine, alanine, and proline, with percentages of (14.15, 11.25, 6.15, 5.62, 4.25, and 4.12)%, respectively. But, a decrease was observed in the amino acids histidine, serine, threonine, valine, lysine, isoleucine, and methionine. Phenylalanine, Glycine, Tyrosine, and Cysteine were found to contain 3.62%, 3.25%, 3.24%, 3.15%, 2.68%, 2.36%, 2.36%, 2.25%, 1.58%, 1.25%, and 1.25%, respectively.

The results show that non-essential amino acids, such as glutamic acid (14.15%) and aspartic acid (6.15%), had the highest percentage. This is attributed to the fact that these acids are involved in the formation of storage proteins, which constitute the largest proportion of seed components, in addition to their role in plant metabolic processes, such as nitrogen transport and glutathione formation. In contrast, essential amino acids appeared in medium to low proportions, with leucine (5.62%) having the highest proportion among essential amino acids, followed by histidine (3.62%) and threonine (3.24%), while arginine had the lowest proportion (1.25%).This limits the total biological value of the protein when used as the sole source of dietary protein. The low proportions of essential amino acids can be explained by the fact that plants, unlike animals, do not have sufficient biosynthetic pathways to efficiently produce all essential amino acids, making them "limiting amino acids" in plant proteins. Lysine and methionine are common limiting amino acids in plant proteins, as evidenced by these results (2.68% and 2.36%, respectively). This is agreed with Cvjetković et al. (2019) reported regarding the distinctive properties of sesame proteins, including their richness in glutamic acid and their relative lack of some essential acids. The researchers indicated that proteins extracted from plant sources such as sesame contain high levels of non-essential acids, especially glutamic acid and aspartic acid, while being limited in some essential acids such as lysine and methionine.

Table (2) Amino Acids in Sesame Meal Powder

N	Amino acid	%
1	Aspartic acid	6.15
2	Glutamic acid	14.15
3	Lysine	2.68
4	Serine	3.25
5	Threonine	3.24
6	Isoleucine	2.36
7	Alanine	4.25
8	Valine	3.15
9	Tyrosine	1.25
10	Arginine	11.25
11	Cysteine	1.25
12	Methionine	2.36
13	Proline	4.12
14	Histidine	3.62
15	Lucien	5.62
16	Phenylalanine	2.25
17	Glycine	1.58

Enzymatic hydrolysis of protein extracted using pepsin

Figure (3) shows the degree of enzymatic hydrolysis of protein extracted using pepsin at different hydrolysis times. The degree of hydrolysis increased with increasing enzyme reaction time until reaching 180 minutes, then decreased again. The degree of hydrolysis reached (19.08, 26.99, 31.08, 37.00, 30.36)% after 30, 60, 120, 180, and 240 minutes of reaction time. The high degree of hydrolysis at the beginning of the enzyme reaction is due to the abundance of peptide bonds that are hydrolyzed by the enzymes. As the reaction progresses, hydrolysis products, such as short peptides and free amino acids, begin to accumulate, causing a gradual decline in enzyme efficiency.The degradation products interfere with the enzyme's active binding sites, or structural changes occur in the substrate as a result of its partial dissociation, which reduces the availability of hydrolyzable bonds and thus reduces the effectiveness of the enzyme's degradation over time. In addition, some proteases have the ability to rebind the degradation products after a period of degradation has passed.

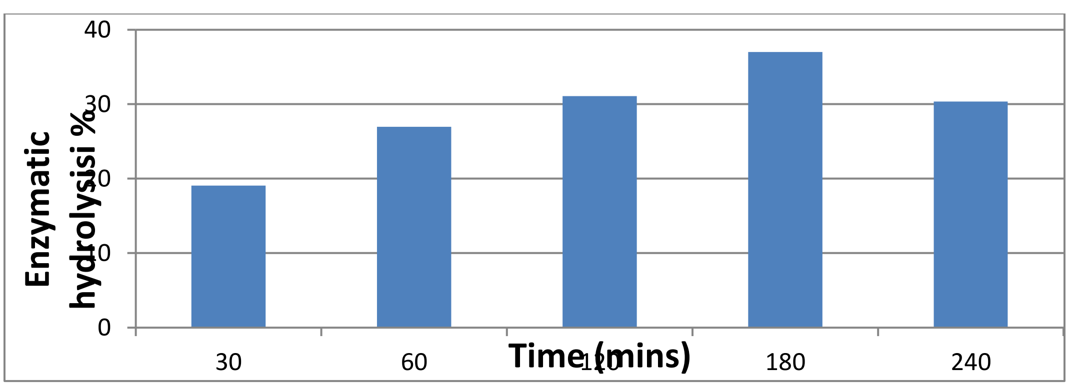


Figure (2) Enzymatic hydrolysis of protein extracted using pepsin

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Kazem (2018) studied the degree of enzymatic decomposition of sesame cake using pepsin. He observed an increase in the degree of decomposition with increasing decomposition time, with the percentage of decomposition reaching (23.25, 28.16, 29.57, 30.74)% after (1, 2, 3, and 4) hours, respectively. As reported by (Sung et al., 2025), the degree of enzymatic hydrolysis of sesame cake using papain increased after 30 minutes of reaction and reached its peak after 4 hours, reaching 67.76%. (Ghanbarinia et al., 2022) also indicated that the efficiency of enzymatic hydrolysis varies depending on the experimental conditions, enzyme type, and hydrolysis time. Enzymes are biocatalysts that accelerate the reaction (Ali et al., 2022).

Estimating the Solubility of Protein Hydrolysates Using Pepsin Enzymes

Figure (3) shows the solubility of protein hydrolysates using pepsin at different pHs ranging from 1 to 12. Solubility increased with increasing hydrolysis time. The highest solubility was recorded at pH 11 and 12, while the lowest solubility was recorded at pH 12. pH 1 and 5, and the decrease in solubility at pH 5, which is the isoelectric point (pI), is due to the decrease in net charge and the resulting electrostatic interactions between protein molecules (Ghorbani et al., 2025).

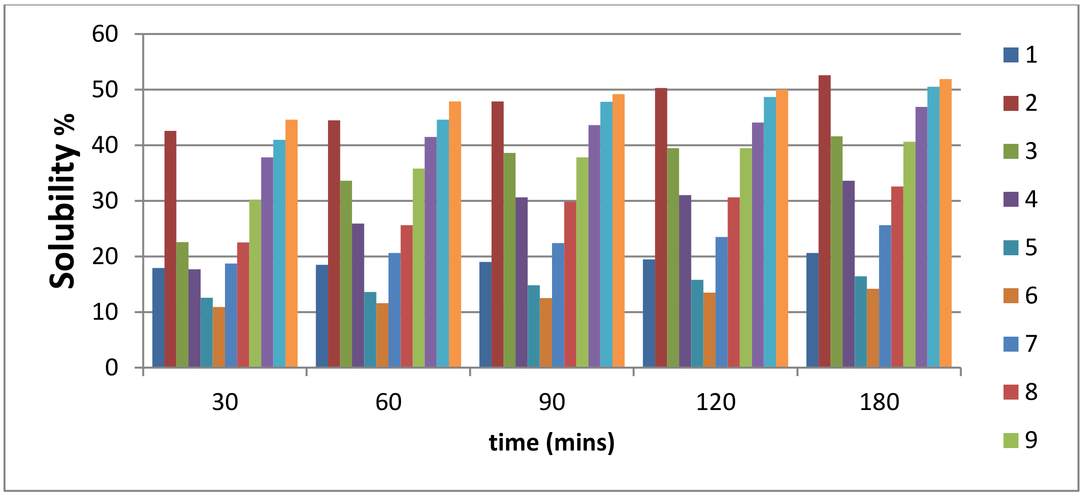

Figure (3) Solubility of Protein Hydrolysates Using Pepsin Enzymes

Noptana et al. (2024) reported that protein solubility increased with increasing hydrolysis time, reaching 32, 36, 38, and 45% after 0.5, 1, 3, and 6 hours, respectively. Protein solubility did not increase when the hydrolysis time increased from 6 **to** 12 hours, indicating that all large, insoluble protein components had been removed by hydrolysis. Noh et al. (2025) reported that protein solubility is affected by the balance of intermolecular repulsive and attractive forces, noting that smaller molecular sizes and smaller, soluble peptides lead to improved solubility.

Estimation of the Water Holding Capacity of Enzymatic Hydrolysates of Sesame Protein

The water holding capacity of the enzymatic hydrolysate of sesame protein using pepsin has been studied (Figure 4). With different pH numbers (5, 6, 7), it is noted that the highest water-holding capacity reached 2.9 g/g at pH 7, while it reached (1.8, 2.4) g/g at pH numbers (5, 6).

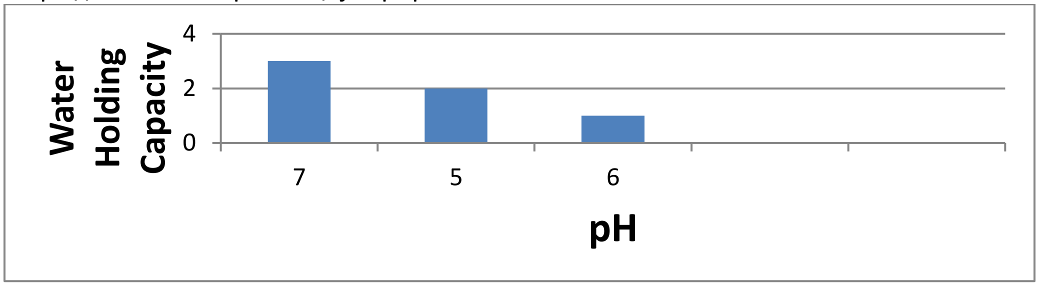

Figure (4) Water Holding Capacity of Enzymatic Hydrolysates of Sesame Protein

Use of pepsin enzyme degraders in the preparation of culture media and the development of microorganisms

The numbers of *B. subtilis* and *S. aureus* reached (167, 150, 158) colony-forming units (CFU), and (190, 185, 205) CFU on commercial solid nutrient media modified with the addition of pepsin enzyme degraders (Table 3), respectively. The numbers of *E. coli* and *S. typhi* grown on commercial MacConkey medium modified with the addition of pepsin enzyme degraders reached (150, 162, 175) and (132, 143, 155) CFU, respectively (Table 4). The results indicate the possibility of using the enzyme hydrolysis products of pepsin extracted from sesame waste as an alternative to the peptone source in the development of the microorganisms used in this study. Ali (2025) obtained good growth results for *E. coli* and *Salmonella typhi* when using fish waste hydrolysates with pepsin instead of a nitrogen source in MacConky agar. Madigan et al. (2018) indicated that microorganisms are affected by osmotic pressure and that the concentration of solutes in the medium can lead to water loss from the cells and inhibit bacterial growth.

Table (3) Numbers of *Staph. aureus* and *B. subtilis* bacteria grown on commercial solid nutrient media and modified media using the nitrogen source of pepsin hydrolysate after 180 and 240 minutes

Culture Medium	Number of bacteria (cfu/ml)×10^5	
	B.Subtilis	*Staph.aureus*
Commercial Solid Nutrient Medium	167	205
Solid Medium Modified with Pepsin Protease after 180 Minutes of Degradation	150	185
Solid Medium Modified with Pepsin Protease after 240 Minutes of Degradation	158	190
LSD	*14.78	*17.05
		(P≤0.05)*

Table (4) Numbers of *E-coli* and *Salmonella typhi* bacteria grown on solid commercial MacConkey medium and modified media using a nitrogen source from the proteolytic enzyme pepsin after 180 and 240 minutes

Culture Medium	Number of bacteria (cfu/ml)×10^5	
	E-coli	*Salmonella typhi*

Commercial Solid Nutrient Medium	175	155
Solid Medium Modified with Pepsin Protease after 180 Minutes of Degradation	150	132
Solid Medium Modified with Pepsin Protease after 240 Minutes of Degradation	162	143
LSD	*21.66	*17.42
(P≤0.05)*		

In the growth results of *S. aureus*, *B. subtillis*, *E. coli*, and *Salmonella typhi*, we note that numbers increase with increasing lysis time. This can be attributed to the provision of free amino acids and easily absorbed peptides, which serve as a rich and rapidly utilizable nitrogen source, as well as improving the properties of the medium.

Aspergillus niger and *Aspergillus flavus* mold growth

The results shows the diameters of *Aspergillus niger* and *Aspergillus flavus* molds on Sabouraud dextrose agar media modified by replacing peptone with proteolytic enzyme pepsin after 180 and 240 minutes of lysis time. The growth diameters reached 530, 525, and 535 mm and 450, 430, and 440 mm, respectively. It is noted that the fungal growth was good compared to growth on the standard medium (Table 5). Diameter of growth of molds *Aspergillus niger* and *Aspergillus flavus* on solid commercial Sabouraud dextrose agar medium and media modified by replacing peptone with the proteolytic enzyme pepsin after 180 and 240 minutes of lysis time.

Culture Medium	Number of mold (cfu/ml)×10^5	
	Aspergillus niger	*Aspergillus flavous*
Sabouraud dextrose agar	535	450
Sabouraud dextrose agar solid medium, modified with pepsin protease after 180 minutes of lysis	525	430
Sabouraud dextrose agar solid medium, modified with pepsin protease after 240 minutes of lysis	530	440
LSD	*12.52	*16.98
(P≤0.05)*		

Ali (2025) demonstrated a significant increase in the growth diameter of *A. niger* and *A. flavus* fungi when using protein hydrolysates of carp fish waste produced by pepsin. He observed that the maximum growth diameter of Aspergillus flavas was 420 mm using the acidic and basic hydrolysates of pepsin after 90 minutes of hydrolysis. The efficiency factor was 0.95, which is lower than the growth diameter of 440 mm in the commercial SDA medium. The maximum growth diameter of *Aspergillus niger* was 530 and 520 mm for the acidic and basic hydrolysates after 90 minutes of hydrolysis. It was noted that the growth diameter of the basic hydrolysate was equal to the growth diameter in the commercial medium.

CONCLUSIONS

The study concludes that sesame meal contains a high percentage of protein, indicating the possibility of preparing protein concentrates with a high protein content from defatted sesame meal powder. The enzymatic hydrolysates possessed high solubility, and the results demonstrated that enzymatic hydrolysates of defatted sesame meal using pepsin are effective nitrogen sources in culture media for the growth of microorganisms.

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